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STIMULATION OF LETTUCE SEED GERMINATION BY ETHYLENE

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DEPARTMENT OF THE ARMY
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ABSTRACT

Ethylene increased the germination of freshly imbibed lettuce (Lactuca sativa L. var. Grand Rapids) seeds. Seeds receiving either red or far-red light or darkness all responded positively to the gas. However, ethylene was apparently without effect on dormant seeds, those that failed to germinate after an initial red or far-red treatment. Carbon dioxide, which often acts as a competitive inhibitor of ethylene, failed to clearly reverse ethylene-enhanced seed germination. While light doubled ethylene production from the lettuce seeds, its effect was not mediated by the phytochrome system since both red and far-red light had a similar effect.

I. INTRODUCTION

The ability of ethylene to increase seed germination has been described in a number of earlier papers.[1-4] In a preliminary report[5] the ability of ethylene to increase lettuce seed germination was described. Grand Rapids lettuce seeds were chosen because it is known[6] that phytochrome is able to regulate the germination of this variety. A number of reports have demonstrated that phytochrome is able to regulate ethylene production from seedling tissue,[5,7,8] and the purpose of our study was to determine if the germination-stimulating action of red light was due to its ability to regulate ethylene production.

II. MATERIALS AND METHODS

Seeds of Lactuca sativa L. var. Grand Rapids were stored at -15 C until required for use. About 100 seeds were spread on three layers of moist filter paper in 9-cm petri dishes and placed in the dark at 30 ± 2 C for a 5-hour imbibition period. After this imbibition period, the seeds were exposed to various light sources or temperatures and then stored either in 10-liter desiccators for ethylene and CO_2 treatments or in gas collection bottles for ethylene production measurements.

The far-red light was provided by a 150-watt incandescent lamp fitted with a 165-mm Corning filter No. 2600 CS 7-69. For red light, Corning filter No. 3961 CS 1-56 was used. All light treatments were given for 15 minutes, which was adequate to elicit the phytochrome response. Except for the exposure to red or far-red light, all manipulations were performed under a green light (Corning filter CS 5-75), which had no effect on seed germination.

Gas chromatography was used to determine the ethylene content of the gas phase surrounding the lettuce seeds. Two-milliliter gas samples were injected into an oxygen-hydrogen flame ionization gas chromatograph fitted with a ¼-inch, 60-cm activated alumina column run at 100 C. Sensitivity of the chromatograph permitted the determination of 25 pl (picoliter = 10^{-12} liter) ethylene per ml. Carbon dioxide used in these experiments contained less than 0.01 ppm ethylene. One ppm of ethylene equals 1.25×10^{-9} g or 1 nl (nanoliter = 10^{-9} liter) ethylene per ml gas phase.

The CO_2 and ethylene were added to the gas phase surrounding the seeds by creating a partial vacuum inside the desiccators, inserting a syringe through a rubber vaccine cap covering the desiccator outlet, and then removing the vaccine cap to equilibrate the contents to atmospheric pressure. Ethylene production was determined by placing the lettuce seeds on moist filter paper in gas collection bottles (5 cm in diameter and 2.5 cm high, fitted with a neck to accommodate a 25-mm diameter rubber vaccine cap) and sampling the gas phase with a syringe.

III. RESULTS

The ability of ethylene to increase the rate of lettuce seed germination is shown in Table 1. The gas increased the rate of germination of seeds given a prior treatment of darkness or red or far-red light.

TABLE 1. EFFECT OF ETHYLENE ON LETTUCE SEED GERMINATION AFTER 24 HOURS

Light Treatment[a]	Germination, %, at Indicated ppm Ethylene			
	0	1	10	100
Far-red	3	4	6	8
Red	33	45	56	56
Dark control	13	22	20	20

a. 500 seeds per treatment.

High concentrations of CO_2 usually block the biological activity of ethylene. However, as shown in Table 2, CO_2 alone increased germination, especially at the 15% concentration, and failed to give a uniform reversal of ethylene action.

Ethylene appears to affect only the initial stages of germination. Figure 1 shows germination was essentially completed by 24 hours and that, while ethylene increased the total per cent germination, the period of time over which germination occurred was not extended.

TABLE 2. EFFECT OF 1 PPM ETHYLENE AND CO_2 ON LETTUCE SEED GERMINATION AFTER 24 HOURS

Light Treatment[a/]	Level of CO_2, %	Germination, %, at Indicated Condition			
		Control	Ethylene	CO_2	CO_2 + Ethylene
Far-red	10	2	8	3	7
Red	10	37	51	36	49
Dark control	10	11	27	21	29
Far-red	15	2	6	4	10
Red	15	37	58	41	48
Dark control	15	2	6	4	10

a. 1,600 seeds per treatment.

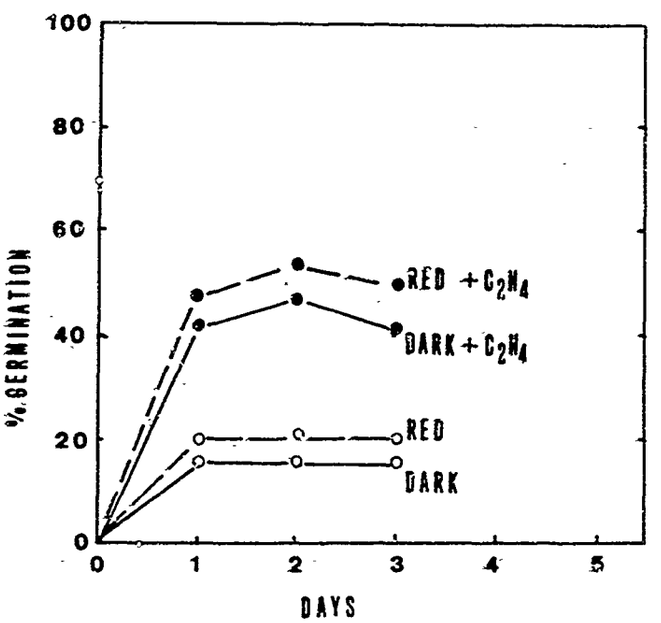

FIGURE 1. Effect of 10 ppm Ethylene Added after Imbibition on the Germination of Grand Rapids Lettuce Seeds.

The ability of ethylene to act only during the initial stages of germination was demonstrated in another way. In this experiment, seeds were permitted to germinate as a result of various light treatments, and after no further germination was observed the remaining seeds were then treated with ethylene. As shown in Figure 2, ethylene had no further effect on the germination of the lettuce seeds. However, the ungerminated seeds were still viable because they germinated when exposed to a 15 C cold treatment (Fig. 3). The cold treatment was applied by lowering the temperature to 15 C after the 3rd day and holding the seeds at that temperature until germination was measured on the 5th day.

Other workers have shown that ethylene production may be controlled by phytochrome.[9,10] Because lettuce seed germination appears to be regulated by phytochrome and ethylene appears to regulate the rate of germination, a series of experiments was initiated to determine if phytochrome regulated ethylene production from the lettuce seeds.

The data in Table 3 indicate that, while light did increase the rate of ethylene production, both red and far-red light appear to be equally effective. This experiment was performed by first giving all the seeds a 5-hour imbibition period at 30 C in the dark followed by a red, far-red, or dark control treatment. The seeds were then allowed to germinate and the germinated seeds were discarded every 24 hours. After 72 hours, when germination was essentially complete, five samples of 200 ungerminated seeds were placed in gas collection bottles. Ethylene production and any further germination was recorded 24 hours later. As shown in Table 3, most of the lettuce seeds remained dormant, and the rate of ethylene production from seeds treated with either red or far-red light was about the same and twice that of the dark controls.

Another way to determine if ethylene production from lettuce seeds is controlled by phytochrome is to compare the rate of ethylene production from germinating seeds given a prior exposure to red, far-red, darkness, and cold. In this experiment all the seeds were given a 5-hour 30 C imbibition period. The seeds were then divided into four sets of 2,000 seeds each. One set was illuminated with 15 minutes of red light; another was illuminated with far-red light. The two other sets of seeds were held in the dark during this time. After this 15-minute treatment period, the seeds from each treatment were placed in gas collection bottles, 100 seeds per bottle, the bottles were sealed, and ethylene production and germination were determined 24 hours later. In the case of seeds treated with cold, the seeds were transferred to a 4 C incubation chamber and left there for 24 hours. Lots of 100 seeds were then placed in gas collection bottles, the bottles were sealed, and germination and the ethylene content of the gas phase were determined 24 hours later. The seeds were kept in the dark during these manipulations.

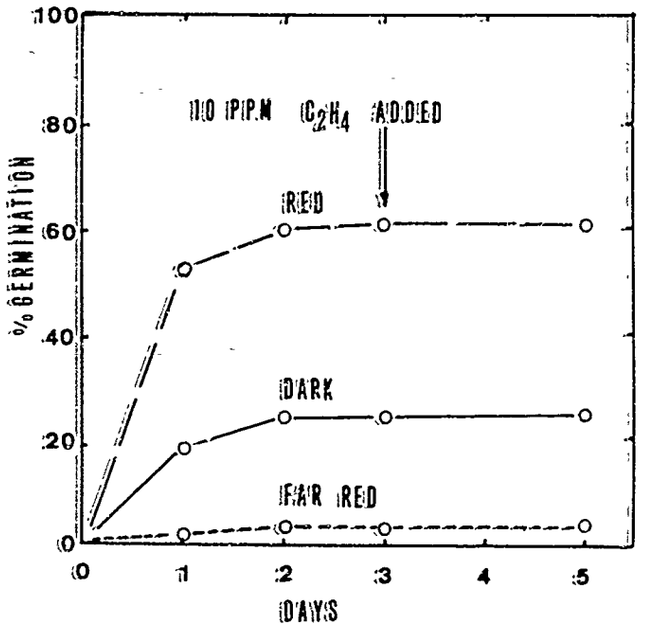

FIGURE 2. Effect of 10 ppm Ethylene Added 3 Days after Imbibition on the Germination of Grand Rapids Lettuce Seeds.

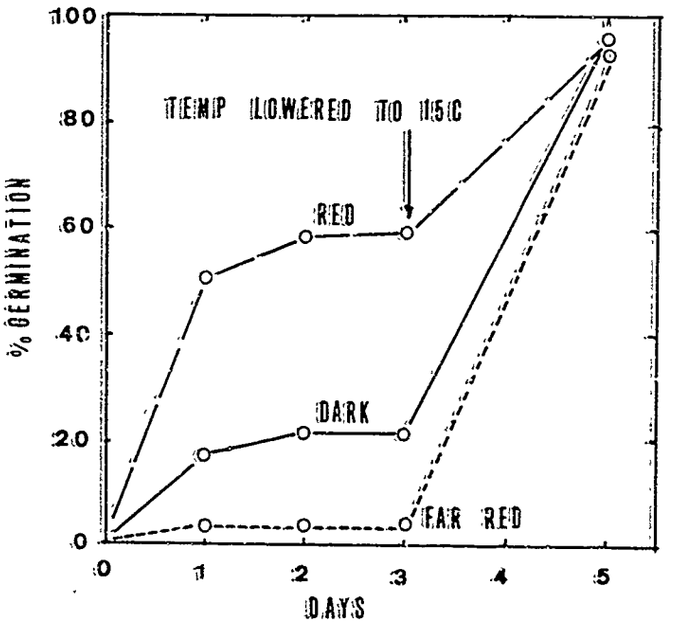

FIGURE 3. Effect of a 15 C Treatment 3 Days after Imbibition on the Germination of Grand Rapids Lettuce Seeds.

TABLE 3. ETHYLENE PRODUCTION FROM NONGERMINATING LETTUCE SEEDS DURING A 24-HOUR PERIOD

Treatment	% Additional Germination	ppm C_2H_4 in Gas Phase	C_2H_4, pl per Nongerminating Seed
Far-red	0.2	0.10	23
Red	0.6	0.094	21
Dark control	0.2	0.044	9.9

The following assumptions were made in calculating the data presented in Table 4. First, that the germination-stimulating 4 C treatment slowed the physiological processes of the seeds to an insignificant rate and that they continued at a normal rate when the seeds were returned to 30 C. Secondly, that the rates of ethylene production calculated for the ungerminated seeds given various treatments described in Table 3 could be used in determining the rates of ethylene production from the germinating seeds. The data in Table 4 indicate that ethylene production from germinating seeds is an order of magnitude greater than that from dormant ones and that light, red or far-red, had no effect on the rate of ethylene production.

TABLE 4. ETHYLENE PRODUCTION FROM GERMINATING LETTUCE SEEDS DURING A 24-HOUR PERIOD

Treatment	Germination, %	ppm C_2H_4 in Gas Phase	C_2H_4, pl per Germinating Seed
Far-red	7	0.090	259
Red	43	0.287	272
Dark control	25	0.145	232
Dark, 4 C	50	0.300	260

IV. DISCUSSION

Stimulation of seed germination by ethylene has been reported earlier by a number of workers.[1-5] Ethylene chlorohydrin, which often mimics ethylene, has also been reported to increase the germination of tree seeds.[11] Data presented here indicate that ethylene could also increase lettuce seed germination. However, CO_2 did not act as a competitive inhibitor as it does in a number of other processes regulated by ethylene, such as root[12] and stem[13] growth inhibition, fruit ripening,[14] celery blanching,[15] flower wilting,[16] abscission,[17] hook opening,[10] and peroxidase formation.[7] Toole, Bailey, and Toole[3] also noticed that CO_2 did not overcome the ability of ethylene to stimulate peanut seed germination. The observation that CO_2 was unable to overcome ethylene-stimulated seed germination suggests that the mechanism of ethylene action in seed germination may be different from other phenomena mentioned above.

The data presented here indicate that ethylene does not act by overcoming dormancy. Treatment of dormant seeds with ethylene had no effect on germination (Fig. 2), but a 15 C cold treatment did result in essentially complete germination (Fig. 3). In addition, ethylene does not appear to act by enhancing phytochrome action; if it had, ethylene would have decreased germination of seeds receiving far-red light as well as increased the germination of seeds receiving red light.

Germination is a complex phenomenon involving imbibition, mobilization, cell enlargement, cell division, and cell differentiation. Ethylene is known to influence a number of these processes, including enzyme secretion,[8] cell enlargement and differentiation,[12] and hook opening.[9,10] The role of ethylene in lettuce seed germination is not clear, but the data presented here suggest that its action is limited to initial steps in the germination process because it was effective only when applied to freshly imbibed seeds (Fig. 1 versus Fig. 2).

Ethylene has been shown to act as an intermediate in the action of auxin,[12,18] gibberellin,[19] abscisic acid,[19] malformin,[20] and 2,4-dichlorophenoxyacetic acid.[21] Goeschl et al.[9] and Kang et al.[10] reported that ethylene may serve as an intermediate in phytochrome-mediated processes such as the hook opening response. Because lettuce seed germination is influenced by ethylene and phytochrome has been shown to regulate germination, experiments on the control of ethylene production from lettuce seeds by red and far-red light were performed. In an abstract published earlier,[5] we reported that phytochrome did regulate ethylene production because seeds treated with red light produced more ethylene than controls. The data presented here, however, indicate that seeds treated with red light produce more ethylene only because germinating seeds produce ten times as much ethylene as dormant ones (Table 3 versus Table 4). While light did appear to increase ethylene production from lettuce seeds, it apparently made no difference if red or far-red light was used (Table 3), and we conclude that the germination-stimulating effect of red light is not due to the control of ethylene production.

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13. ABSTRACT

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14. Key Words
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*Germination
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